Quantikine® ELISA

Human IFN-β Immunoassay

Catalog Number DIFNB0

For the quantitative determination of human Interferon beta (IFN-β) concentrations in cell culture supernates.

This package insert must be read in its entirety before using this product.
For research use only. Not for use in diagnostic procedures.

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INTRODUCTION

Interferon beta (IFN-β), also known as fibroblast IFN, is a secreted, approximately 22 kDa member of the type I interferon family of molecules (1). Mature human IFN-β shares 47% and 46% amino acid sequence identity with the mouse and rat proteins, respectively. Fibroblasts are the major producers of IFN-β, but it can also be produced by dendritic cells, macrophages, and endothelial cells in response to pathogen exposure (2). It is transcriptionally regulated by TRAF3, IRF3, IRF7, and NFκB (3, 4). It has also been shown that the RIPK1 and RIPK3 kinases play a role in LPS-induced upregulation of IFN-β in mice (5). Following secretion, IFN-β signals through the heterodimeric IFN-α/β Receptor and activates the JAK/STAT signaling pathway (6-9). IFN-β appears to have a complex role in the regulation of inflammasomes. It has been shown to directly inhibit NLRP1 and NLRP3 inflammasomes in a STAT1-dependent manner and increase the susceptibility of mice to *C. albicans* infection (10). In contrast, *L. monocytogenes* has been shown to activate the NLRP3 inflammasome in an IFN-β-dependent manner (11). Viral infection of human mini-gut organoids induces IFN-β which leads to upregulation of Viperin and IFIT1 IFN-stimulated genes (12).

IFN-β-deficient mice show increased susceptibility to experimental autoimmune encephalomyelitis (EAE), a disease model of human multiple sclerosis (MS) (13). Furthermore, IFN-β has been shown to suppress the Th17 cell response in both MS and EAE and has commonly been used as a treatment for MS (14-18). Low levels of IFN-β have been associated with the hyporesponsive state of monocytes from sepsis patients, suggesting that IFN-β may have a role in restoring monocyte function and reversing immunosuppression (19). Type I IFNs (IFN-α and IFN-β) appear to have a role in the polarization of neutrophils in cancer. Inflammation, along with functional type I IFN signaling, was shown to alter neutrophil polarization towards anti-tumor phenotype (20).

The Quantikine® Human IFN-β Immunoassay is a 4.5 hour solid phase ELISA designed to measure human IFN-β in cell culture supernates. It contains CHO cell-expressed recombinant human IFN-β and has been shown to accurately quantitate the recombinant factor. Results obtained using natural human IFN-β showed linear curves that were parallel to the standard curves obtained using the Quantikine® kit standards. These results indicate that this kit can be used to determine relative mass values for natural human IFN-β.

PRINCIPLE OF THE ASSAY

This assay employs the quantitative sandwich enzyme immunoassay technique. A monoclonal antibody specific for human IFN-β has been pre-coated onto a microplate. Standards and samples are pipetted into the wells and any IFN-β present is bound by the immobilized antibody. After washing away any unbound substances, an enzyme-linked monoclonal antibody specific for human IFN-β is added to the wells. Following a wash to remove any unbound antibody-enzyme reagent, a substrate solution is added to the wells and color develops in proportion to the amount of IFN-β bound in the initial step. The color development is stopped and the intensity of the color is measured.

LIMITATIONS OF THE PROCEDURE

- FOR RESEARCH USE ONLY. NOT FOR USE IN DIAGNOSTIC PROCEDURES.
- The kit should not be used beyond the expiration date on the kit label.
- Do not mix or substitute reagents with those from other lots or sources.
- Samples, controls, and standards must be pipetted within 15 minutes.
- If samples generate values higher than the highest standard, further dilute the samples with calibrator diluent and repeat the assay.
- Any variation in diluent, operator, pipetting technique, washing technique, incubation time or temperature, and kit age can cause variation in binding.
- Variations in sample collection, processing, and storage may cause sample value differences.
- This assay is designed to eliminate interference by other factors present in biological samples. Until all factors have been tested in the Quantikine® Immunoassay, the possibility of interference cannot be excluded.

TECHNICAL HINTS

- When mixing or reconstituting protein solutions, always avoid foaming.
- To avoid cross-contamination, change pipette tips between additions of each standard level, between sample additions, and between reagent additions. Also, use separate reservoirs for each reagent.
- To ensure accurate results, proper adhesion of plate sealers during incubation steps is necessary.
- When using an automated plate washer, adding a 30 second soak period following the addition of Wash Buffer, and/or rotating the plate 180 degrees between wash steps may improve assay precision.
- Substrate Solution should remain colorless until added to the plate. Keep Substrate Solution protected from light. Substrate Solution should change from colorless to gradations of blue.
- Stop Solution should be added to the plate in the same order as the Substrate Solution. The color developed in the wells will turn from blue to yellow upon addition of the Stop Solution. Wells that are green in color indicate that the Stop Solution has not mixed thoroughly with the Substrate Solution.

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human IFN-β Microplate	898542	96 well polystyrene microplate (12 strips of 8 wells) coated with a monoclonal antibody specific for human IFN-β.	Return unused wells to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 1 month at 2-8 °C.*
Human IFN-β Standard	898544	2 vials of recombinant human IFN-β in a buffered protein base with preservatives; lyophilized. *Refer to the vial label for reconstitution volume.*	Use a new standard for each assay. Discard after use.
Human IFN-β Conjugate	898543	21 mL of a monoclonal antibody specific for human IFN-β conjugated to horseradish peroxidase with preservatives.	May be stored for up to 1 month at 2-8 °C.*
Assay Diluent RD1-19	895467	11 mL of a buffered protein base with preservatives.	
Calibrator Diluent RD5-4	895435	21 mL of a concentrated buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	21 mL of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Color Reagent A	895000	12 mL of stabilized hydrogen peroxide.	
Color Reagent B	895001	12 mL of stabilized chromogen (tetramethylbenzidine).	
Stop Solution	895032	6 mL of 2 N sulfuric acid.	
Plate Sealers	N/A	4 adhesive strips.	

* Provided this is within the expiration date of the kit.

OTHER SUPPLIES REQUIRED

- Microplate reader capable of measuring absorbance at 450 nm, with the correction wavelength set at 540 nm or 570 nm.
- Pipettes and pipette tips.
- Deionized or distilled water.
- Squirt bottle, manifold dispenser, or automated microplate washer.
- 500 mL graduated cylinder.
- Horizontal orbital microplate shaker (0.12" orbit) capable of maintaining a speed of 500 ± 50 rpm.
- Test tubes for dilution of standards and samples.
- Human IFN-β Controls (optional; R&D Systems®, Catalog # QC243).

PRECAUTIONS

The Stop Solution provided with this kit is an acid solution.

Some components in this kit contain a preservative which may cause an allergic skin reaction. Avoid breathing mist.

Color Reagent B may cause skin, eye, and respiratory irritation. Avoid breathing fumes.

Wear protective gloves, clothing, eye, and face protection. Wash hands thoroughly after handling. Refer to the MSDS on our website prior to use.

SAMPLE COLLECTION & STORAGE

The sample collection and storage conditions listed below are intended as general guidelines. Sample stability has not been evaluated.

Cell Culture Supernates - Remove particulates by centrifugation and assay immediately or aliquot and store samples at ≤ -20 °C. Avoid repeated freeze-thaw cycles.

REAGENT PREPARATION

Bring all reagents to room temperature before use.

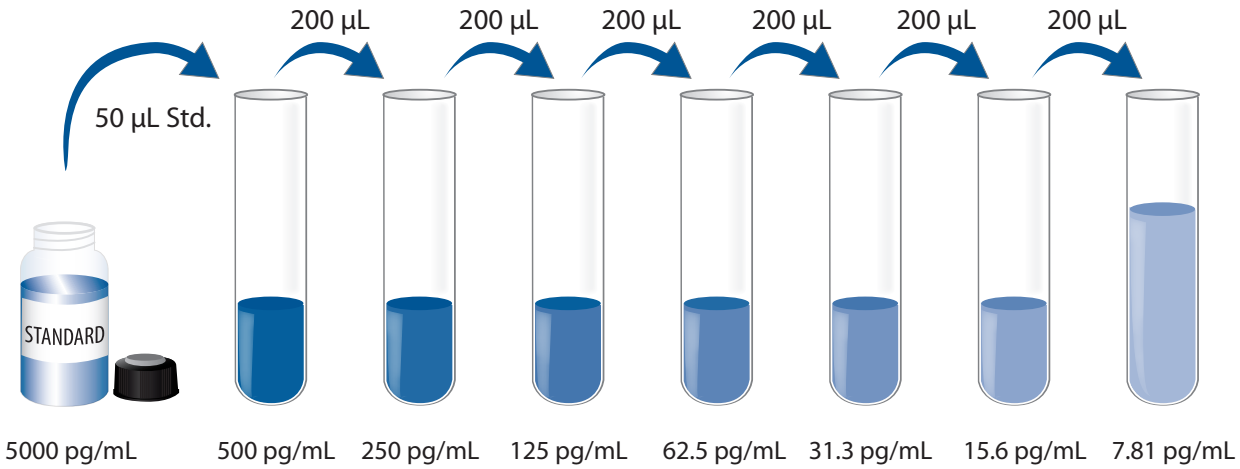
Wash Buffer - If crystals have formed in the concentrate, warm to room temperature and mix gently until the crystals have completely dissolved. Add 20 mL of Wash Buffer Concentrate to deionized or distilled water to prepare 500 mL of Wash Buffer.

Substrate Solution - Color Reagents A and B should be mixed together in equal volumes within 15 minutes of use. Protect from light. 200 µL of the resultant mixture is required per well.

Human IFN-β Standard - **Refer to the vial label for reconstitution volume.** Reconstitute the Human IFN-β Standard with deionized or distilled water. This reconstitution produces a stock solution of 5000 pg/mL. Mix the standard to ensure complete reconstitution and allow the standard to sit for a minimum of 5 minutes with gentle agitation prior to making dilutions.

Note: *Standards must be used within 45 minutes of reconstitution.*

Pipette 450 µL of Calibrator Diluent RD5-4 into the 500 pg/mL tube. Pipette 200 µL into the remaining tubes. Use the stock solution to produce a dilution series (below). Mix each tube thoroughly before the next transfer. The 500 pg/mL standard serves as the high standard. Calibrator Diluent RD5-4 serves as the zero standard (0 pg/mL).



ASSAY PROCEDURE

Bring all reagents and samples to room temperature before use. It is recommended that all standards, samples, and controls be assayed in duplicate.

1. Prepare all reagents, working standards, and samples as directed in the previous sections.
2. Remove excess microplate strips from the plate frame, return them to the foil pouch containing the desiccant pack, and reseal.
3. Add 50 µL of Assay Diluent RD1-19 to each well.
4. Add 50 µL of standard, control, or sample per well. Cover with the adhesive strip provided. Incubate for 2 hours at room temperature on a horizontal orbital microplate shaker (0.12" orbit) set at 500 rpm ± 50 rpm. A plate layout is provided to record samples and standards assayed.

   Note: *Standard, control, and samples must be pipetted within 15 minutes.*
5. Aspirate each well and wash, repeating the process three times for a total of four washes. Wash by filling each well with Wash Buffer (400 µL) using a squirt bottle, manifold dispenser, or autowasher. Complete removal of liquid at each step is essential to good performance. After the last wash, remove any remaining Wash Buffer by aspirating or decanting. Invert the plate and blot it against clean paper towels.
6. Add 200 µL of Human IFN-β Conjugate to each well. Cover with a new adhesive strip. Incubate for 2 hours at room temperature on the shaker.
7. Repeat the aspiration/wash as in step 5.
8. Add 200 µL of Substrate Solution to each well. Incubate for 30 minutes at room temperature **on the benchtop. Protect from light.**
9. Add 50 µL of Stop Solution to each well. The color in the wells should change from blue to yellow. If the color in the wells is green or the color change does not appear uniform, gently tap the plate to ensure thorough mixing.
10. Determine the optical density of each well within 30 minutes, using a microplate reader set to 450 nm. If wavelength correction is available, set to 540 nm or 570 nm. If wavelength correction is not available, subtract readings at 540 nm or 570 nm from the readings at 450 nm. This subtraction will correct for optical imperfections in the plate. Readings made directly at 450 nm without correction may be higher and less accurate.

CALCULATION OF RESULTS

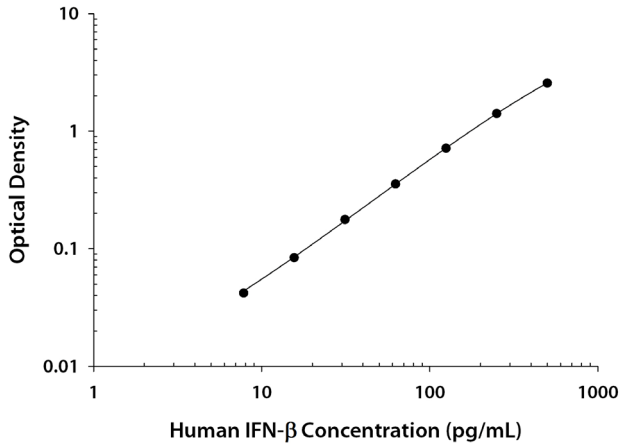
Average the duplicate readings for each standard, control, and sample and subtract the average zero standard optical density (O.D.).

Create a standard curve by reducing the data using computer software capable of generating a four parameter logistic (4-PL) curve-fit. As an alternative, construct a standard curve by plotting the mean absorbance for each standard on the y-axis against the concentration on the x-axis and draw a best fit curve through the points on the graph. The data may be linearized by plotting the log of the human IFN-β concentrations versus the log of the O.D. and the best fit line can be determined by regression analysis. This procedure will produce an adequate but less precise fit of the data.

If samples have been diluted, the concentration read from the standard curve must be multiplied by the dilution factor.

TYPICAL DATA

This standard curve is provided for demonstration only. A standard curve should be generated for each set of samples assayed.



(pg/mL)	O.D.	Average	Corrected
0	0.019 0.020	0.020	—
7.81	0.062 0.062	0.062	0.042
15.6	0.103 0.104	0.104	0.084
31.3	0.196 0.197	0.197	0.177
62.5	0.374 0.376	0.375	0.355
125	0.725 0.745	0.735	0.715
250	1.390 1.471	1.431	1.411
500	2.581 2.596	2.589	2.569

PRECISION

Intra-assay Precision (Precision within an assay)
Three samples of known concentration were tested twenty times on one plate to assess intra-assay precision.

Inter-assay Precision (Precision between assays)
Three samples of known concentration were tested in twenty separate assays to assess inter-assay precision. Assays were performed by at least three technicians using two lots of components.

	Intra-Assay Precision			Inter-Assay Precision		
Sample	1	2	3	1	2	3
n	20	20	20	20	20	20
Mean (pg/mL)	58.3	159	295	56.4	160	275
Standard deviation	1.71	6.01	13.8	5.26	13.0	21.9
CV (%)	2.9	3.8	4.7	9.3	8.1	8.0

RECOVERY

The recovery of human IFN-β spiked to levels throughout the range of the assay in various matrices was evaluated.

Sample Type	Average % Recovery	Range
Cell culture media (n=5)	107	95-111%

LINEARITY

To assess the linearity of the assay, samples containing high concentrations of human IFN-β were serially diluted with calibrator diluent to produce samples with values within the dynamic range of the assay.

		Cell culture supernates (n=6)
1:2	Average % of Expected	95
	Range (%)	90-100
1:4	Average % of Expected	96
	Range (%)	92-102
1:8	Average % of Expected	96
	Range (%)	91-102
1:16	Average % of Expected	98
	Range (%)	90-105

SENSITIVITY

Twenty assays were evaluated and the minimum detectable dose (MDD) of human IFN-β ranged from 0.269-0.781 pg/mL. The mean MDD was 0.480 pg/mL.

The MDD was determined by adding two standard deviations to the mean O.D. value of twenty zero standard replicates and calculating the corresponding concentration.

CALIBRATION

This immunoassay is calibrated against a highly purified CHO cell-expressed recombinant human IFN-β produced at R&D Systems®.

The NIBSC/WHO IFN-β 3rd International Standard 00/572 (Human, rDNA, Glycosylated) was evaluated in this kit. The dose response curve of the reference reagent 00/572 parallels the Quantikine® standard curve. To convert sample values obtained with the Quantikine® Human IFN-β kit to approximate NIBSC/WHO 00/572 Units, use the equation below.

NIBSC/WHO (00/572) approximate value (IU/mL) = 0.43 x Quantikine® Human IFN-β value (pg/mL)

Note: *Based on data generated in June 2017.*

SAMPLE VALUES

Cell Culture Supernates:

A549 human lung carcinoma cells were cultured in Kaighn's Nutrient Mixture F-12 supplemented with 10% fetal bovine serum until nearly confluent. The cells were cultured unstimulated or stimulated with 10 µg/mL of poly I:C in the presence of Lipofectamine 2000 (LF2K) for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-β.

Condition	pg/mL
Unstimulated	ND
Stimulated	848

ND=Non-detectable

CCD-1070Sk human foreskin fibroblast cells were cultured in MEM NEAA Earle's Salts supplemented with 10% fetal bovine serum until nearly confluent. The cells were cultured unstimulated or stimulated with 10 µg/mL of poly I:C in the presence of Lipofectamine 2000 (LF2K) for 24 hours. Aliquots of the cell culture supernates were removed and assayed for levels of human IFN-β.

Condition	pg/mL
Unstimulated	ND
Stimulated	2323

ND=Non-detectable

SPECIFICITY

This assay recognizes natural and recombinant human IFN-β.

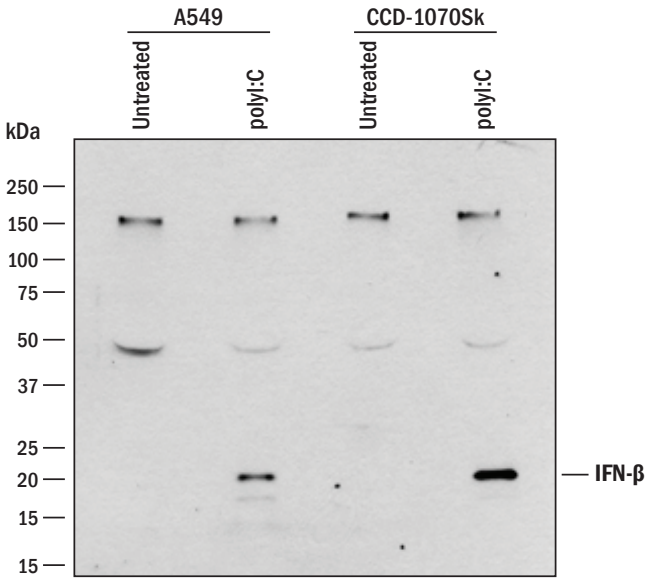
The factors listed below were prepared at 50 ng/mL in calibrator diluent and assayed for cross-reactivity. Preparations of the following factors at 50 ng/mL in a mid-range recombinant human IFN-β control were assayed for interference. No significant cross-reactivity or interference was observed.

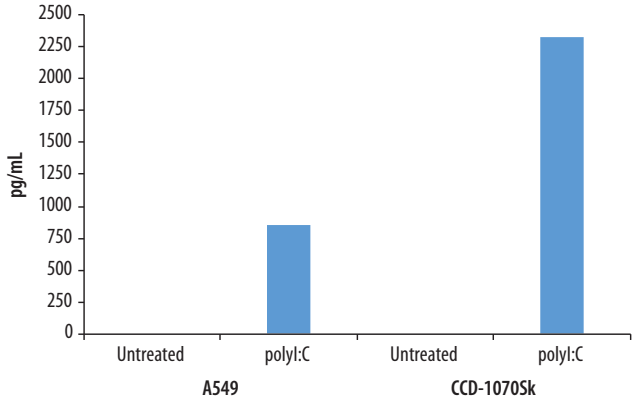
Recombinant human:

IFN-α2
IFN-α/β R1
IFN-α/β R2
IFN-γ
IKKα
IL-28A
IL-28B
IL-29

Recombinant mouse:

IFN-β1





Conditioned media samples were analyzed by Western Blot and Quantikine® ELISA. Human A549 and CCD-1070Sk cells were left untreated, or treated with 10 µg/mL of polyI:C in the presence of LF2K for 24 hours prior to harvest. For Western Blot, samples were resolved under reducing SDS-PAGE conditions, transferred to a PVDF membrane, and immunoblotted with goat anti-human IFN-β (R&D Systems®, Catalog # AF814). The Western Blot shows a direct correlation with ELISA value for these samples.

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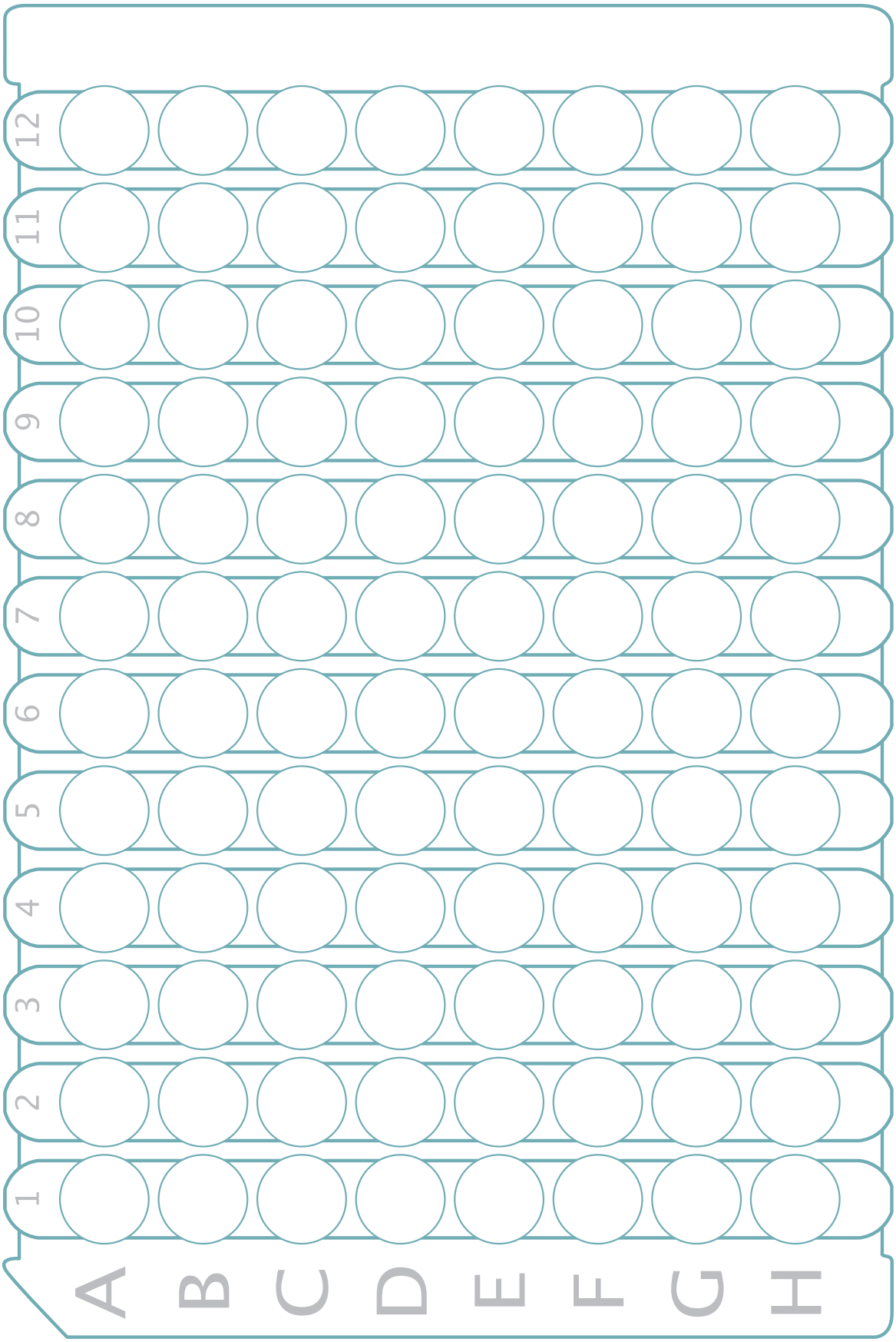
PLATE LAYOUT

Use this plate layout to record standards and samples assayed.



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